RESEARCH PAPER

The Histological Effect of Lepidium sativum Extract on the Mammary Glands in Female Rabbits

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ABSTRACT

Background: The mammary glands are essential for milk production and supporting the growth of newborns, as their tissue condition reflects the level of physiological activity and hormonal changes in the body.

Objective: This study was carried out to investigate the effect of Lepidium sativum on the development and growth of the mammary glands in female rabbits during two physiological stages: virgin and lactating.

Materials and methods: A total of 20 sexually mature female domestic rabbits were randomly allocated into 4 categories, each one consisting of 5 pets, and were selected based on 2.5 to 3 kg weights, divided into two main groups. Group 1: Non-pregnant, non-lactating rabbits, and included Section A1 (control): were given sterile water. Section B1 was treated with L. sativum extract powder at a dose of 200 mg/kg body weight. Group 2: Lactating rabbits, Section A2 (control), were treated with sterile water. Section B2 was treated with L. sativum extract at a dose of 200 mg/kg body weight. The experiment lasted for three weeks. Measurements Prolactin by ELISA kit, and with a histological examination of the mammary gland.

Results: The results indicated a significant increase in prolactin levels when treated with L. sativum in non-lactating rabbits from (7.80 ± 0.47) to (29.00 ± 0.21) ng/ml and in lactating rabbits from (40.50 ± 0.21) to (62.60 ± 0.54) ng/ml. L. sativum significantly increased mammary gland duct diameter in non-lactating rabbits from (11.28 ± 0.12) to (26.44 ± 0.32) micron and in lactating rabbits from (52.67 ± 0.54) to (64.69 ± 0.65) micron. Histological examinations showed clear differences between the groups. In G1, section A, the mammary gland appeared inactive, dominated by connective tissue, with few ducts and small primary alveoli. In G1 section B, a marked increase in the number of ducts and the beginning of alveolar formation was observed, with slight dilation and the presence of secretory material in some ducts. G2 section A appeared to be in an active lactation state, with large secretory alveoli within dense glandular tissue separated by thin connective tissue. G2 section B recorded the highest degree of activity, with alveoli appearing more developed and denser compared to the other groups.

Keywords: Lepidium sativum, mammary glands, Prolactin, Histological study

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Introduction

Traditional uses of medicinal plants and their associated properties have been documented by several authors. One of the medicinal plants that has been reported to have multi-system effects and also possess important biological activities on reproductive characteristics (Shah et al., 2021)**.** Garden cress, or *Lepidium sativum*, is an annual vegetarian plant with several traditional therapeutic uses. This plant is distinguished by its small top leaves and lobed lower leaves, as well as by its potent, fragrant scent. Its bioactive constituents, which are used in various cultures to cure diseases and also improve health generally, offer it special importance. the potential effects on endocrine action, particularly on the pituitary and breasts. Its bioactive agents may intervene with the function and structure of mammary and pituitary glands (Kamani et al., 2017)**.** Several pharmacological properties of Lepidium sativum have been recognized, such as anti-inflammatory, antioxidant, and hormone-modulating actions (Aboul Naser et al., 2024)**.**

The mammary gland is a compound, branched tubule alveolar structure and a major characteristic of

mammals. There are two main types of epithelium in the mammary gland, namely, luminal and basal (Biswas et al., 2022b). Mammary glands produce milk as a source of nutrition for supporting the postnatal survival of offspring for reproductive success in all mammals (Stead et al., 2022). The fatty stroma is the supportive network for the bi-layered epithelium structure and provides nutrients, blood supply, and immune defenses besides the physical structure to the gland. The major components of stromal connective tissues are adipocytes, fibroblasts, vascular endothelial cells, a variety of innate immune cells (both macrophages and mast cells), and nerves. The fatty stroma is the supportive network for the bi-layered epithelium structure and provides nutrients, blood supply, and immune defenses besides the physical structure to the gland (Urbaniak, 2016).

The current study attempted to assess the effect of *Lepidium sativum* extract on the mammary glands in adult local female rabbits by measuring the diameter of the duct in the mammary gland, the level of prolactin hormone for all groups, and the study of histological tissue.

Materials and Method

Preparation of ethanolic extract of *Lepidium sativum*: The work was conducted in the laboratories of Al-Ameed University using the dried plant material of wild arugula (Eruca sativa). The alcoholic extract was prepared by weighing 50 g of the dried plant and grinding it using a household herb grinder to obtain a fine powder. The powder was then placed in a glass flask, and 500 mL of ethanol at 70% concentration was added. The mixture was left at room temperature for a specified period with intermittent stirring, and then it was filtered to obtain the extract. The yield of the dried extract was 3.3%, equivalent to 3.3 g of extract per 50 g of dried plant material. The active compounds in the extract were identified using the technique GC-mass spectroscopy.

Animal Experimental: This study was conducted for the period from the beginning of one month in 2025 to the end of the second month in 2025, with 20 adult females who were randomly allocated into four categories, each one consisting of 5 pets whose weights ranged from 2.5 to 3 kg and ages ranged from 9 to 13 months. And we're housed and maintained in the animal house/College of Veterinary Medicine/University of Kerbala. Animals were placed in special cages with lighting 12 hours a day and good ventilation; the floor was furnished with soft sawdust, and care was taken to maintain the cleanliness of the cages and change the floor constantly and sterilize it with disinfectants, as well as continuous care for the cleanliness of irrigation bottles and the shelter room, providing animals with water and a standard diet freely throughout the duration of breeding and research. Rabbits were left to acclimatize for three weeks before the start of the experiment**.**

Experimental Design: The animals were randomly divided into two main groups, each consisting of 10 adult female rabbits, as follows:

1-First Group (G1): Non-pregnant, Non-lactating Mature Females This group was subdivided into two subgroups (5 animals per subgroup):

A- Control Group (A1): Received only standard feed and clean drinking water,

B- Treatment Group (B1): Received L.S. extract orally, once daily for three consecutive weeks, at a dose of 200 mg/kg body weight (Vazifeh et al., 2022)**.**

2- Second Group (G2): Lactating Females After natural mating and parturition, this group of females entered the lactation phase. On the first day of lactation, the group was divided into two subgroups (5 animals per subgroup):

A- Control Group (A2): Received standard diet and water only during the lactation period.

B- Treatment Group (B2): Received L.S. extract orally, once daily for three consecutive weeks during lactation, at the same dose used in the first group.

Blood Sample Collection: After the end of the experiment, the animals were transferred to the Kerbala University / College of Veterinary Medicine for the purpose of completing the procedures for withdrawing blood and dissecting the animals to obtain the organs required in the experiment. The transfer process was carried out over 3 weeks, which is the oral dosing period. The animals were weighed after the experiment and then anesthetized by using chloroform anesthetic. After ensuring that the animals were anesthetized, blood was withdrawn directly from the rabbit's ear using a sterile medical 5 ml syringe to obtain the largest amount of blood. The blood samples were placed directly in sterile test tubes free of anticoagulant gel tubes, and then the tubes were transferred to a centrifuge at a speed of 3000 rpm for 15 minutes to obtain the serum, which is transferred to small Eppendorf tubes and clean, dry, labeled tubes. The serum is stored in the refrigerator at a low

temperature of -20°C until the biochemical and histopathological tests are performed.

Hematological Study

Prolactin test: Hormonal concentrations such as prolactin were quantified using ELISA kits based on the solid-phase sandwich enzyme-linked immunosorbent assay principle (DRG instruments, Germany). Each well of the microplate was precoated with a monoclonal antibody that targets a specific epitope of the respective hormone. Patient samples were added to the wells along with an enzyme-conjugated antibody linked to horseradish peroxidase (HRP). Following incubation, unbound materials were washed off. The bound enzyme produced a colorimetric reaction upon addition of the substrate, and the color intensity was directly proportional to the hormone concentration, as measured spectrophotometrically at 450 nanometers (Bioscienlces, 2019).

Histological study of mammary glands

Histomorphology examinations for cell shape Comparison of all samples, Cell diameter,

Statistical analysis: The Statistical Packages of Social Sciences (SPSS) program (2019) was used to detect the effect of different groups in study parameters. The t-test was used to compare significant differences between means in this study.

Results and Discussion

GC-mass analysis: Table 1 showed the results of gas chromatography coupled to mass spectrometry (GC-MS). The high peaks are due to compounds present in high concentrations produced by *Lepidium sativum*. Several chemicals identified were analogous between the extracts.

Sl.no	Name of compounds	Molecular formula	M.W(g\mol)	Peak area %
1	D-Limonene	C10H16	4.280	4.10
2	2-Propanol, 1,1'-oxybis-	C6H14O3	4.606	4.18
3	1-Propanol, 2-(2-hydroxypropoxy)	C6H14O	4.926	5.20
4	1,6-Octadien-3-ol, 3,7-dimethyl	C11H18O	5.122	1.71
5	1,6-Octadien-3-ol, 3,7-dimethyl	C11H18O	5.383	2.19
6	Benzyl nitrile	C6H5CH2CN	5.813	1.25
7	Benzene, 1-isocyano-2-methyl-	C8H7N	6.230	37.76
8	4H-Pyran-4-one, 2,3-dihydro-3,5-di	C6H8O4	6.413	3.12
9	. alpha.-Terpineol	C10H18O	6.987	1.06
10	Butane, 1,1'-oxybis [3-methyl-	C12H26O3	7.333	0.79
11	Linalyl acetate	C12H20O2	7.731	0.58
12	Benzene, (isothiocyanate methyl)-	C8H7NS	9.752	1.34
13	Benzene acetic acid, cyclopentolate es	C13H16O2	9.844	0.96
14	2-Hydroxy-1-(1'-pyrrolidiyl)-1-	C8H13NO	12.172	1.29
15	cis-Stilbene	C12H14O4	12.876	3.68
16	Diethyl Phthalate	C12H14O4	13.229	3.84
17	2- [2- [2- [2-(2,2,3,3,3-Pentafluoro	C3H3F5O	14.285	0.77
18	Propane, 1,1,3,3-tetraethoxy-	C11H24O	14.716	0.65
19	Octanal, 2-(phenylethylene)-	C15H20O	15.492	6.24
20	(2,5-Dioxo-2,5-dihydropyrrol-1-yl)	$C_{11}H_6N_2O_2$	15.811	1.25
21	Dibutyl phthalate	$C_6H_4(CO_2C_4H_9)_2$	18.179	0.93
22	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	18.427	2.42
23	hexadecenoic acid, ethyl ester	$C_{18}H_{36}O_2$	18.609	0.67
24	Ethylene brassylate	C15H26O4	18.942	0.65

25	**Phytol**	**C20H40O**	**20.024**	**0.76**
26	**9,12,15-Octadecatrienoic acid**	**C 18 H 30 O 2**	**20.592**	**6.81**
27	**9,12,15-Octadecatrienoicacid, ethyl ester**	**C 20 H 34 O 2**	**20.670**	**2.52**
28	**Ethyl 9,12,15-octadecatrienoate**	**C 20 H 34 O 2**	**20.781**	**1.28**
29	**Methyl 8,11,14-heptadecatrienoate**	***C18H30O2***	**20.970**	**1.08**
30	**1-(2-Hydroxymethyl-pyrrolidin-1yl**	**C7H13NO2**	**21.772**	**0.91**

Table 1. GC-MS result of *Lepidium sativum* extract

Our study, using gas chromatography/mass spectrometry (GC/MS), revealed a range of biologically active compounds in the Lepidium sativum extract, most notably unsaturated fatty acids such as alpha-linolenic acid (ALA), as well as phenolic compounds and flavonoids known for their hormonal regulatory properties. Alpha-linolenic acid is one of the compounds that has demonstrated anti-inflammatory effects by inhibiting the expression of inflammatory cytokines (IL-6, IL-1β, TNF-α), which may contribute to creating a more stable physiological environment within the body. This balance in the internal milieu is considered a contributing factor in stimulating the hypothalamic-pituitary axis, which in turn regulates prolactin secretion (Al Asmari, 2015).

On the other hand, the presence of flavonoids and phytoestrogens in the extract may mimic or enhance the effect of natural estrogen, one of the most important hormones that stimulate prolactin secretion from the anterior pituitary gland (Ali et al., 2024). These compounds bind to estrogen receptors in the hypothalamus, inhibiting the release of dopamine, the primary inhibitory neurotransmitter that controls prolactin secretion. With the decrease in dopamine's inhibitory effect, lactotroph cells in the pituitary gland increase their secretion of prolactin, which is consistent with the findings that showed a gradual increase in prolactin levels in the extract-treated groups, particularly after birth (Jaffar et al., 2024).

Thus, the dual effect of the extract compounds—by inhibiting inflammation and stimulating estrogenic pathways—could explain the significant increase in prolactin levels we observed in rabbits. This effect not only increases hormone concentrations but may also contribute to enhancing pituitary function and linking it to the physiological needs of the postpartum period, when prolactin secretion is essential for preparing the mammary glands and supporting lactation (Babaeiyazdi et al., 2022).

Hematological prolactin: Table (2) showed the levels of prolactin hormone in the rabbit groups. The results indicate that there were statistically significant differences in the prolactin level. In non-lactating rabbits (Group 1), the prolactin level increased in (section B) treated with *L. sativum*, recording 29.00 ± 0.21 ng/ml compared to (section A), the control group, which recorded 7.80 ± 0.47 ng/ml. Similarly, in lactating rabbits (Group 2), the prolactin level increased in (section B) treated with *L. sativum*, recording 62.60 ± 0.54 ng/ml, compared to (section A), where the control group recorded 40.50 ± 0.21 ng/ml.

Section	**Mean ± SD of Prolactin (ng/ml)**		**T-test (P-value)**
	Group 1	**Group 2**	
Section A	**7.80 ±0.47**	**40.50 ±0.21**	**3.509 ** (0.0001)**
Section B	**29.00 ±0.21**	**62.60 ±0.54**	**5.378 ** (0.0001)**
T-test (P-value)	**4.391 ** (0.0001)**	**6.173 ** (0.0001)**	**---**
**** (P<0.01).**			

Table 2: Comparison between difference groups in prolactin hormones level in rabbits

The results of the statistical analysis indicated a statistically significant difference at $p < 0.01$ in prolactin levels among the studied rabbit groups, with the control group recording the lowest level compared to the groups treated with the extract (*Lepidium sativum*). The typical reference range for prolactin

levels in normal female rabbits is around 2 to 20 ng/ml (Abdel-Khalek et al., 2022). However, these values can vary based on factors such as age and time of day. This gradual increase in levels indicates that the extract stimulates the secretion of prolactin, a key hormone responsible for stimulating the mammary glands and milk production. Its effect was also more pronounced after birth than before birth, suggesting a synergy between the natural physiological changes of birth and the active compounds in the extract (Zhang et al., 2021). This is attributed to the richness of *Lepidium sativum* seeds in plant compounds such as phytoestrogens and flavonoids, which may activate the hypothalamic-pituitary-prolactin axis, which explains the gradual rise in the hormone, reaching its highest levels in the treated group after birth. The results of the current study are consistent with other studies (Moustafa et al., 2025) that indicated the role of active compounds in extracts in raising prolactin levels.

Diameter of Duct: Table (3) showed the mammary gland duct diameter measurements in the rabbit groups. The results indicate statistically significant differences between the groups ($P<0.01$). In non-lactating rabbits (Group 1), the mammary gland diameter in section B, which was treated with *L. sativum*, increased to 26.44 ± 0.32 micron compared to section A (control group), which recorded 11.28 ± 0.12 micron. Similarly, in lactating rabbits (Group 2), the diameter in section B treated with *L. sativum* was 64.69 ± 0.65 micron, while section A (control group) recorded 52.67 ± 0.54 micron. These findings suggest that *L. sativum* supplementation enhanced mammary gland development, with a more pronounced effect observed in lactating rabbits due to the physiological demands of milk production.

Section	Mean ± SD of Mammary gland measurement diameter (Micron)		T-test (P-value)
	Group 1	Group 2	
Section A	11.28 ±0.12	52.67 ±0.54	4.760 ** (0.0001)
Section B	26.44 ±0.32	64.69 ±0.65	6.154 ** (0.0001)
T-test (P-value)	3.115 ** (0.0001)	4.294 ** (0.0001)	---
** (P<0.01).			

Table 3. Mammary gland diameter of duct

The results of the current study showed that the diameter of the mammary glands increased across groups from (G1) to (G2). This variation reflects a gradual increase in the expansion of the milk ducts across groups, while the stability of the cell number indicates that the changes were mainly related to an increase in diameter without a difference in the numbers of epithelial cells.

The study results indicate that the diameter of the mammary ducts was clearly affected by the treatment received by the rabbits in each group. The first group (control), which received only sterile water, had the smallest duct diameter, reflecting the normal condition of the gland in the absence of any stimulating factors (Vasiu et al., 2023). In contrast, the second group, treated with *Lepidium sativum* extract, showed a significant increase in diameter, indicating that the extract contributed to a relative expansion of the mammary ducts. This is likely due to the stimulation of growth or functional activity of the gland through its biologically active components, such as phytoestrogens (Petrine & Del Bianco-Borges, 2021).

The third group, the postpartum group, had a significantly larger diameter compared to the first and second groups. This is expected given the physiological changes that accompany the postpartum period, when the gland's secretory activity increases in response to hormonal changes associated with lactation. While the fourth group, the postpartum group treated with *Lepidium sativum* extract, recorded the highest diameter of the milk ducts, indicating a cumulative effect of both the postpartum physiological state and the effect of the plant extract, which further enhanced the dilation of the ducts (Ranade & Mudgalkar, 2021). The results of the current study are consistent with a study conducted by (Moronkeji et al., 2024) that indicated the dilation of the mammary

glands by the effect of the extract of *Vernonia amygdalina*.

Histological Results of mammary glands

G1 (section A1) (control): This histological slide showed the mammary gland state stained with H&E. In the nonpregnant, nonlactating mammary gland, the tissue is predominantly composed of dense connective tissue (stroma) with scattered ducts and only small or rudimentary alveoli. The stroma contains abundant collagen fibers, fibroblasts, and blood vessels. The glandular components are relatively sparse compared to the lactating state, as shown in Figure 1.

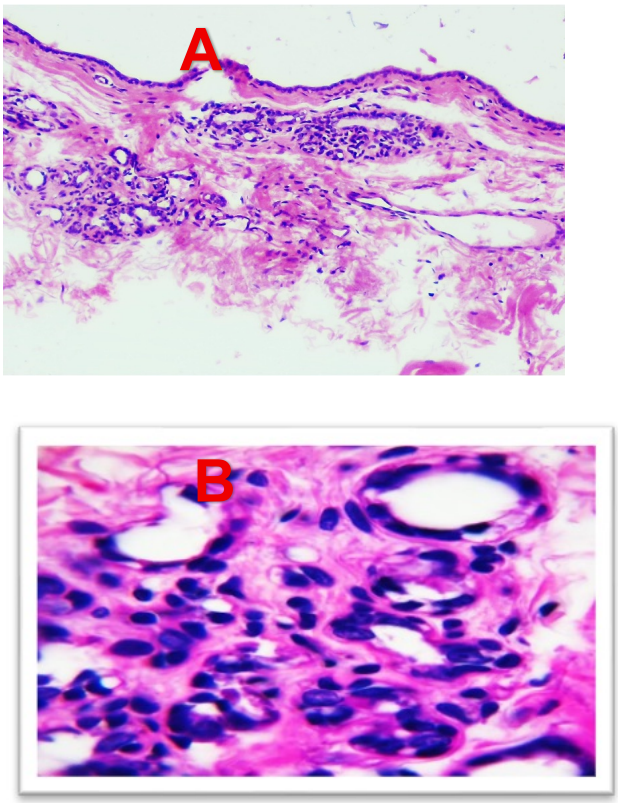

Figure (1): Histological section mammary gland, alveoli lobule (black arrow), alveoli (red arrow), intralobular duct (yellow arrow), H&E stain, A. 100X, B. 1000X

G1 (section B1): This histological slide shows the mammary gland after administration of *Lepidium sativum;* it demonstrates clear structural differences compared to the normal resting mammary gland G1 (section A1). The glandular component is more pronounced compared to the inactive state. The connective tissue stroma is still present but appears less dominant due to the increased proliferation of ducts and alveolar-like structures. Multiple ducts and developing alveoli are evident, with increased number and density compared to the resting slide. The ductal lumina are wider in some places, often containing eosinophilic secretory material, as shown in Figure 2.

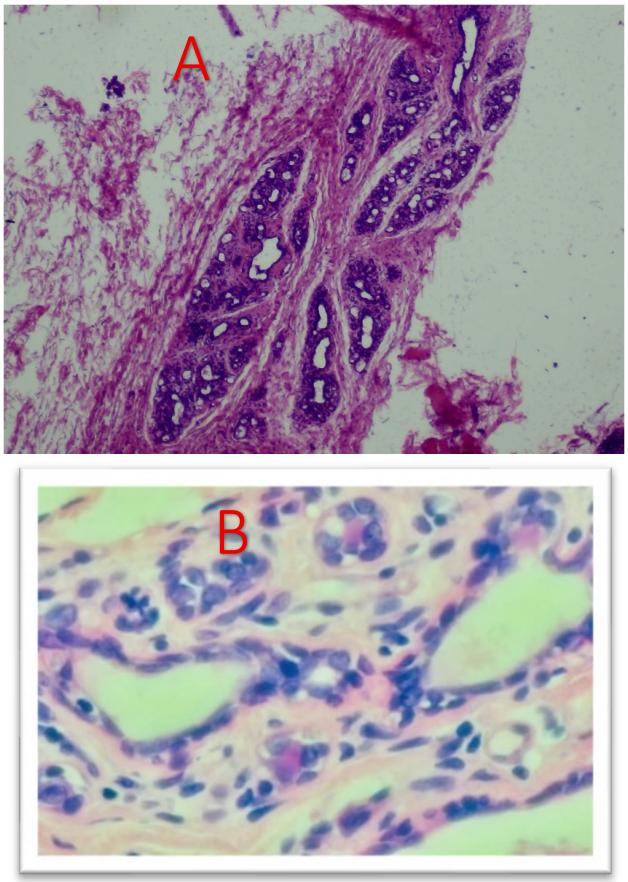

Figure 2: histological section mammary gland, enlargement alveoli lobule (black arrow), wide diameter intralobular duct (yellow arrow), increase secretory cell (red arrow), H&E stain, A.100X, B.1000X,

G2 (section A2): Histological examination of the mammary gland after 21 days in the control group during lactation shows the alveoli are moderately enlarged and rounded, with variable diameters. Many alveolar lumens are distended with secretory material, indicating functional activity. The epithelial lining of alveoli is cuboidal to low columnar, in some areas flattened due to pressure from accumulated secretion. The ducts are clearly visible, wide, and irregular in shape, larger than the alveoli. Their lumens contain

eosinophilic secretory material. The duct epithelium appears mostly cuboidal, but in dilated ducts it can be flattened. Branching ducts connect with multiple surrounding alveoli. The interlobular connective tissue is reduced in amount compared to the alveolar tissue. Septa are present but relatively thin and wide, allowing for expansion of glandular elements. The stroma consists of loose connective tissue rich in fibroblasts and blood vessels. Figure 3

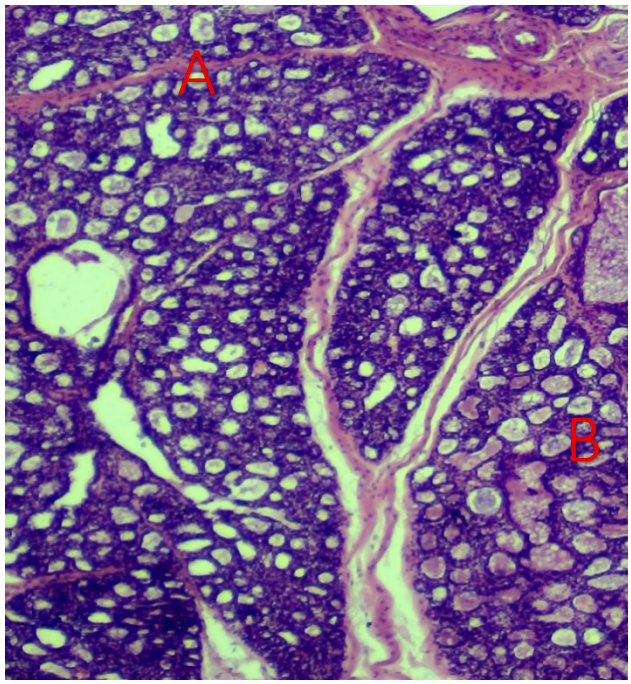

Figure 3: Histological section mammary gland in G2 section A2, alveoli branching (red arrow), active alveoli (yellow arrow), inactive alveoli (black arrow), lactocyte cell (green arrow), H&E stain, A. 100X, B. 1000X

G2 (section B2): Histological examination of the mammary gland after administration of *L. sativum* (garden cress) extract for 21 days shows the alveoli are large, rounded to polygonal, and highly numerous, occupying most of the tissue section. Their lumens are expanded and filled with secretory material, reflecting active milk production, with cells showing enlarged cytoplasm and prominent nuclei. Compared to normal lactation, the alveoli appear more numerous and more distended. The secretory epithelial cells are moderately hypertrophied with clear granular cytoplasm. Some cells appear slightly vacuolated due to accumulation of milk components. The ducts are more numerous and clearly dilated, with well-defined wide lumens. Many ducts contain consistent milk secretions. The branching of the duct system is apparent, joining multiple groups of alveoli. The interlobular connective tissue is markedly reduced, being thin and less prominent compared to non-lactating tissue. It mainly consists of delicate strands of loose connective tissue with blood vessels to support the secretory activity. Hypertrophied secretory epithelial cells with active secretion. Large, numerous alveoli filled with milk product. Dilated, branching ducts with accumulated secretions. Reduced connective tissue stroma, allowing expansion of secretory units. Figure 4

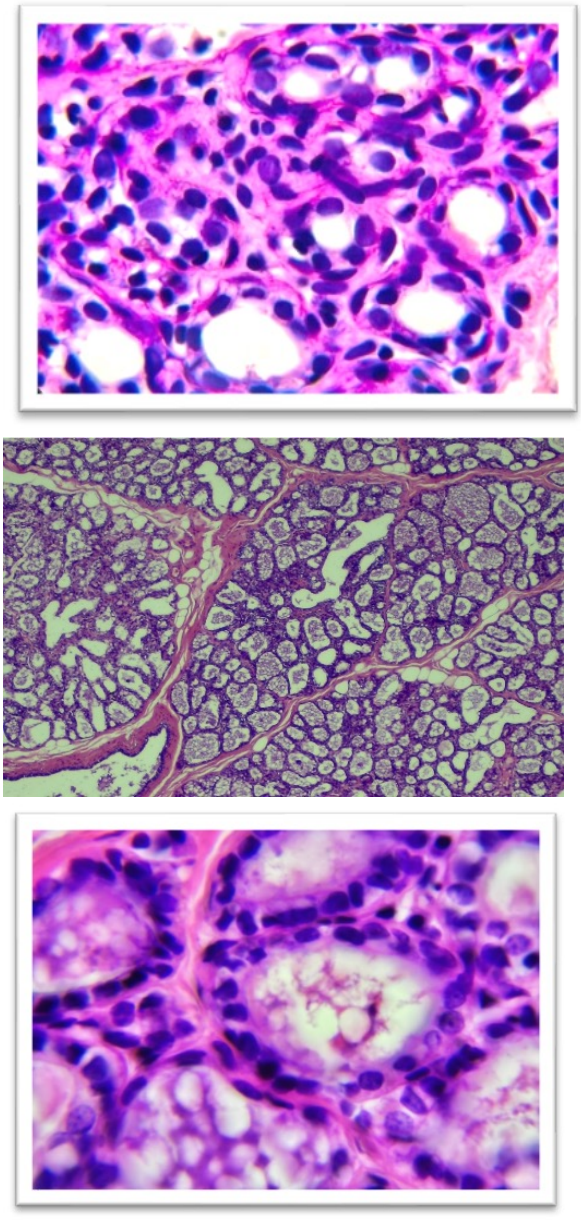

Figure 4: G2 section B, Histological section mammary gland, increase dimeter of interlobular excretory duct (yellow arrow), increase number and size of alveoli (red arrow), increase number of secretory cell (black arrow), H&E stain 100X, B.1000X,

Histological changes in the mammary gland indicate that *Lepidium sativum* extract has a stimulating effect on the growth and development of glandular tissue by increasing epithelial cell proliferation, activating ductal branching, and forming secretory vesicles (Jena et al., 2023). This is likely due to its content of bioactive compounds such as phytoestrogens and flavonoids, which act in a manner similar to estrogen and progesterone, enhancing the binding of these compounds to estrogen receptors in the glandular tissue, thereby stimulating growth and differentiation (Rispo et al., 2024). The extract may also contribute to raising prolactin levels, which is directly responsible for stimulating lactation and increasing the secretory activity of glandular cells. Several studies have supported these findings, such as (Zhang et al., 2021) study, which showed that administering *Lepidium sativum* extract to rats resulted in a significant increase in the size and number of secretory vesicles and an increase in prolactin levels. (Biswas et al., 2022a) study also demonstrated that the extract helped stimulate structural growth of the mammary gland and improve milk production. However, some studies have indicated that the intensity of this effect may vary depending on the dose, the duration of administration, and the animal's physiological condition. Some research has shown that low doses may not achieve the same degree of stimulation, while very high doses may lead to hormonal imbalance, reducing the effectiveness of the effect. This explains the discrepancy in results between different studies (Abdella & Khalifah, 2021).

Conclusion:

Results of the present study demonstrate that *Lepidium sativum* extract had a stimulating role in increasing the dilatation of the mammary ducts both in non-pregnant rabbits and in the postpartum period, but the effect was more pronounced in the postpartum state, indicating a positive interaction between the normal physiological changes after birth and the effect of the extract, which enhanced the activity of the mammary gland and led to a significant increase in diameter.

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